

Toxopathological Studies on Ethylene Bisdithiocarbamates Metabolite in Albino Rats

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ABSTRACT

The carcinogenic effect of ETU on albino rats was studied using 500 ppm of ETU for 90 days. Hematological and serum biochemical pictures and histopathology were recorded at 7, 30 and 90 days of experiment.

The experimental rats showed nausea and decrease body weight at 7 days. While at 30 and 90 days post-application (PA), alopecia and marked decrease in body weight were seen.

Significant decreases in RBCs, Hb, PCV% and WBC values were noticed after 30 days PA of the ETU. The chemistry parameters showed significant increase in levels of ALT, AST, BUN, CRN, T-CHO, F-CHO, Na⁺, PL, TG and TSH at 30 days PA of the ETU till the end of the experiment. While the levels of TP, Ca⁺, PL, K⁺, ALB, T3 and T4 were significantly decreased after 30 days PA of the ETU.

Grossly, the thyroid glands and internal organs were enlarged and nodulated. Microscopically, at 7 days PA of the ETU hepatic hemorrhage, cholangitis, acute focal interstitial nephritis and round cells infiltration in the gastric and intestinal mucosa were evident. At 30 days PA of the ETU, hepatocytes fatty change, coagulative necrosis in renal tubules, squamous cell papilloma in non glandular part of the stomach and mucinous degeneration in the intestine were seen. At 90 days PA of the ETU, adenomacarcinomas in the thyroid and gastric glands, fatty change and coagulative necrosis in the liver, interstitial nephritis in the kidneys and catarrhal enteritis were evident.

The ethylene bisdithiocarbamates metabolite ETU, has toxic effects in rats and possible carcinogenicity of these agents in humans are expected. Therefore, further studies should be done to assess this risk in human.

KEYWORDS: toxicological, histopathological, biochemistry, ETU, rat.

INTRODUCTION

Dithiocarbamates are widely used as fungicides because of their efficacy against a broad spectrum of fungi and their associated plant diseases. Dithiocarbamates are also used in industry as slimicides in water-cooling systems, in sugar, pulp, and paper manufacturing, and as vulcanization accelerators and antioxidants in rubber. Because of their chelating properties, they are also used as scavengers in waste-water treatment. Dithiocarbamates can be divided into two groups: the ethylenbisdithiocarbamates (EBDC), such as maneb, zineb, and mancozeb, and the dimethyldithiocarbamates (DMDC), including ferbam, ziram, and thiram. Ethylenethiourea (ETU) is one of the principal metabolites of EBDCs and is thought to be the source of most of the toxicity associated with EBDCs. ETU is a degradation by-product of the manufacture of EBDCs (in tobacco, cooked foods, commercial beverages, etc.), formed during product storage [1, 2, 3, 4].

EBDCs are absorbed primarily dermally and are metabolized to ethylene thiourea (ETU). Dermal absorption of EBDCs range from 1 to 10%, and approximately 7.5% of the absorbed dose is converted to ETU [5].

Ethylene thiourea (2 mercaptoimidazoline) has been used as an accelerator in the rubber industry since 1948. No hazard to health from this material was recorded until 1969 when liver tumours were reported in mice after the oral administration of 215mg/kg [6]. In 1972, the induction of thyroid carcinoma in rats and hypoplastic and simple goitre was reported after the oral administration of 350 ppm of Ethylene thiourea [7]. The International Agency for Research in Cancer of the World Health Organisation [8] has evaluated the carcinogenic risk of ethylene thiourea (ETU) to man and classified it as an antithyroid substance and as a suspect carcinogen, since this type of substance is believed to induce thyroid tumours through the suppression of thyroxin synthesis leading to hyperplasia of the thyroid gland.

Animal studies using several mammalian species showed that, ETU is rapidly absorbed from the gastrointestinal tract and cleared from the body. [9], noticed ETU after only 5 min in the blood of rats administered an oral dose of 100 mg 14C-ETU/kg body weight. Within 48 hr, 82–99% of the oral dose was eliminated via urine and about 3% was eliminated via faeces. Another study found that, approximately 70% of

an oral dose of ETU was eliminated in urine and 1% in faeces [10, 11]. Comparable results were found for mice, whereas in monkeys 55% was eliminated via urine within 48 hr and < 1.5% was eliminated via faeces [12]. ETU and its metabolites have been found to have a half-life of about 28 hr in monkeys, 9–10 hr in rats, and 5 hr in mice [13].

Increased incidences of thyroid carcinomas and hepatomas have been observed in rats and mice orally exposed to ethylene thiourea [14].

The present study aimed to evaluate the carcinogenic effect of ethylene thiourea (ETU) through experimental exposure via monitoring the hematological, serum biochemical and pathological findings.

MATERIALS AND METHODS

Experimental animals:

A total of sixty clinically healthy male albino rats of 100 ± 5 gm body weight were brought from Fac. Vet. Med., Zagazig University. These rats were grouped into 2 equal groups (each of 30 rats) and reared in metal cages under hygienic condition, maintained on a balanced diet and provided with tap water along the period of experiment.

Chemical:

Ethylenethiourea (ETU) was purchased from Sigma Aldrich Chemicals Company, Germany (99% pure).

Experimental design:

Rats in group 1 served as a control along the period of experiment. Rats in group 2 were fed on a basal diet provided with 500 ppm of ethylene thiourea (ETU) for 90 days. The dose was selected based on previous study [15].

Clinical signs and gross lesions:

Clinical signs were monitored throughout the study. The animals were observed for morbidity, and mortality at least twice daily. Animal weights, detailed clinical observations, and food consumption were determined weekly until study termination. At necropsy, the gross lesions were recorded in thyroid, liver, kidney, intestine and stomach of experimented rats.

Hematological and serum biochemical parameters:

Blood samples were collected from each animal in both groups via retro-orbital venous plexus at 7, 30 and 90 days post treatment. Each sample was divided into two parts, first portion was collected in vacutainer tubes containing EDTA, as anticoagulant for hematological studies (RBC, Hb, APTT, WBC and PCV%) according to the standard techniques described by [16]. The second portion was collected in a plain centrifuge tubes and serum was separated and used for determine the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), the levels of total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (CR), phospholipid (PL), triglyceride (TG), total cholesterol (T-CHO), free cholesterol (F-CHO), calcium (Ca), inorganic phosphorus (Pi), sodium (Na) and potassium (K), using an Auto Clinical Analyzer, Hitachi Model 7150 (Hitachi Ltd., Tokyo, Japan). T3/T4 and TSH levels in serum were measured by RIA using analytical kits.

Histopathological technique:

Tissue specimens were taken from the thyroid, liver, kidney, stomach and intestine of experimented rats (groups 1 & 2), at 7, 30 and 90 days of experiment and fixed in 10% phosphate buffer formalin. Processed routinely and blocked in paraffin then, five micron thick paraffin sections were prepared and stained with hematoxylin and eosin, (H&E) [17].

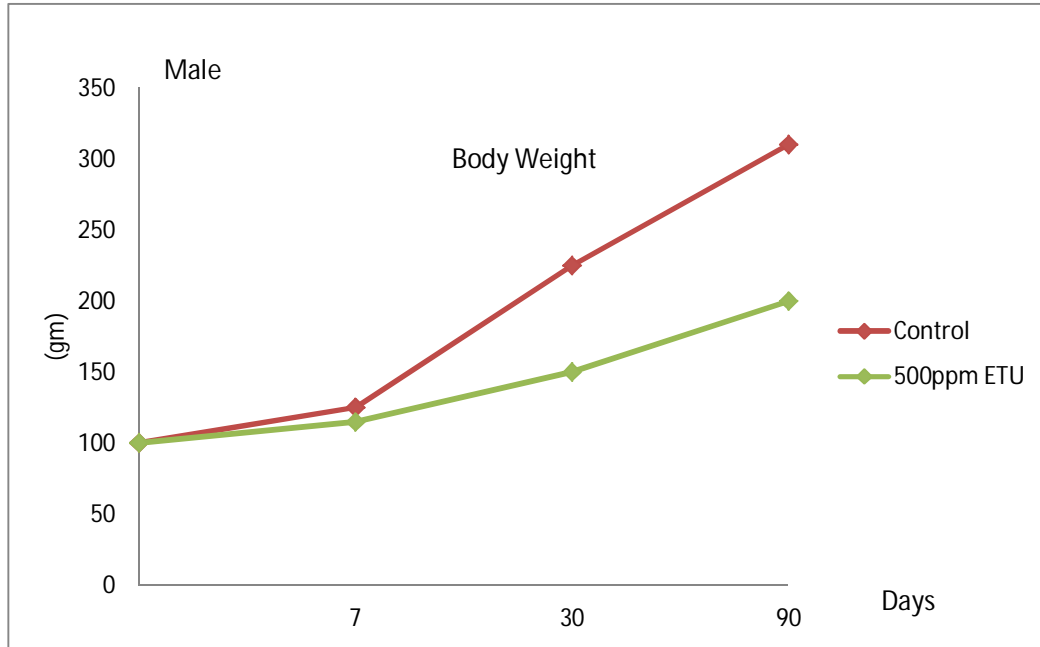
Statistical analyses:

The obtained data were statistically analyzed using SAS program [18]. All quantitative data, were statistically analyzed by one-way analysis of variance (ANOVA) techniques with Dunnett's. Significance was calculated where the $p < 0.05$ level.

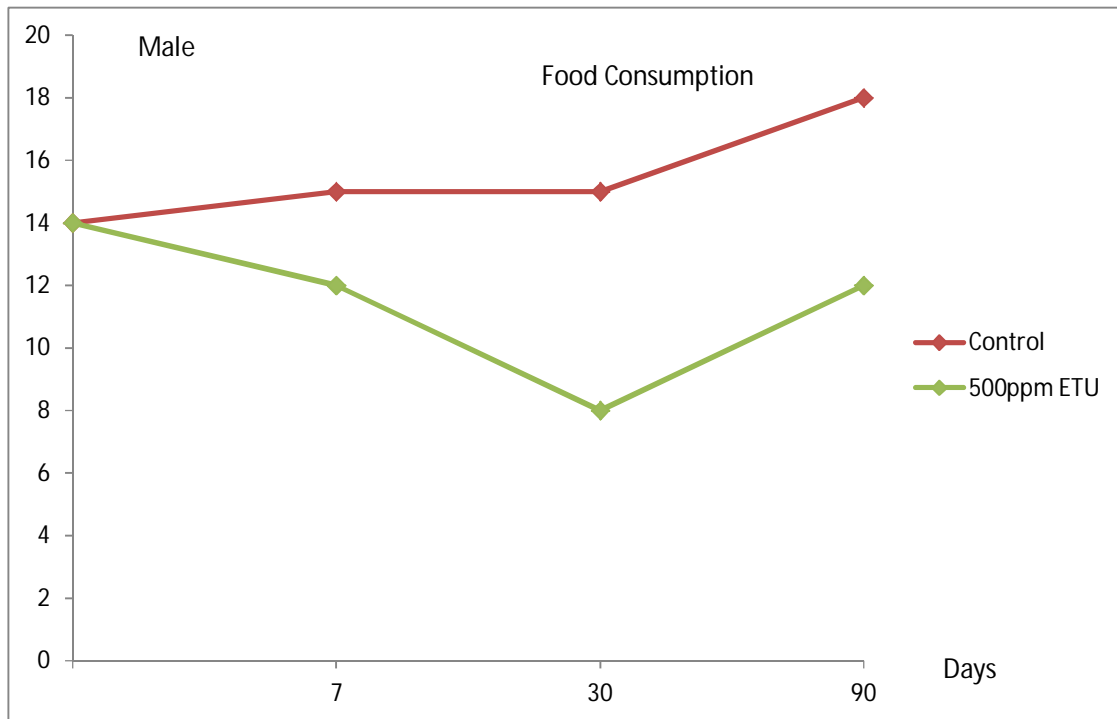
RESULTS

Clinical signs:

No mortality among experimented rats due to ETU exposure occurred, the experimental rats showed nausea, ruffled or easily removed hair and decrease body weight gain and food consumption at 7 days. At 30 and 90 days of the experiment alopecia, unbalanced movement, emaciation and severe suppression in body weight gain with decreased food consumptions were seen (curves 1 & 2).



Curve 1.Body weight for male rats post administration of ETU for 7, 30 and 90 days in comparison to the control.



Curve 2.Food consumption for male rats post administration of ETU for 7, 30 and 90 days in comparison to the control.

Hematological findings:

The hematological parameters in this study were summarized in table (1), a significant decreases in RBCs count, Hb concentration, PCV% and WBC values were seen with an increase in the partial thromboplastin time (APTT, blood clotting time) at 30 days PA of the ETU to the experimented rats till the end of the experiment.

Table 1. Some hematological parameters in rats post administration of ETU for 7, 30 and 90 days in comparison to the control (Mean values \pm SE).

parameters Time of sampling	RBCs ($10^6/\mu\text{l}$)	Hb (gm/dl)	PCV (%)	WBCs ($10^3/\mu\text{l}$)	APTT (sec)
Control	8.54 \pm 0.36	14.8 \pm 0.62	46.18 \pm 2.08	6.84 \pm 0.29	26.8 \pm 1.13
7 days	8.35 \pm 0.43	14.2 \pm 0.88	44.13 \pm 2.74	6.22 \pm 0.39	27.04 \pm 1.68
30 days	7.12 \pm 0.43*	12.6 \pm 0.76*	40.25 \pm 1.81*	5.55 \pm 0.33**	32.23 \pm 1.93*
90 days	6.82 \pm 0.44**	11.8 \pm 0.76**	36.6 \pm 2.34**	5.43 \pm 0.35**	40.06 \pm 2.56**

* Significant at $P < 0.05$

** Highly significant at $P < 0.01$.

Clinical biochemistry findings:

The chemistry parameters are summarized in table (2). It was recognized that at 30 days PA of the ETU to the experimented rats till the end of the experiment, a significant increase in the levels of ALT, AST, BUN, CRN, T-CHO, F-CHO, Na⁺, PL, TG and TSH were observed but significantly decrease in the levels of TP, Ca⁺, PI, K⁺. ALB, T3 and T4 were seen.

Table 2. Some Biochemical parameters in rats post administration of ETU for 7, 30 and 90 days in comparison to the control (Mean values \pm SE).

Time of sampling Parameters	Control	7 days	30 days	90 days
AST (U/L)	87.2 \pm 4.80	92.09 \pm 5.09	105.93 \pm 6.36*	121.6 \pm 8.88**
ALT (U/L)	49.1 \pm 2.70	50.95 \pm 2.96	61.29 \pm 3.43*	73.41 \pm 4.62**
TP (g/dl)	6.98 \pm 0.38	6.78 \pm 0.42	5.81 \pm 0.40*	5.25 \pm 0.37**
Alb (g/dl)	4.15 \pm 0.23	4.02 \pm 0.25	4.12 \pm 0.28	3.55 \pm 0.22**
T-CHO (mg/dl)	88.12 \pm 3.79	92.69 \pm 5.75	150.52 \pm 10.39**	179.55 \pm 10.83**
F-CHO (mg/dl)	21.33 \pm 1.17	24.15 \pm 1.21	28.08 \pm 1.80*	47.33 \pm 2.89**
TG (mg/dl)	48.91 \pm 2.69	50.26 \pm 2.61	59.44 \pm 3.27*	66.17 \pm 4.17**
PL (mg/dl)	157.03 \pm 7.07	166.12 \pm 8.63	193.33 \pm 7.72**	213.12 \pm 11.29**
BUN (mg/dl)	20.32 \pm 1.12	22.55 \pm 1.24	25.89 \pm 1.68*	31.09 \pm 1.96**
Cr (mg/dl)	0.96 \pm 0.05	1.02 \pm 0.05	1.43 \pm 0.07**	1.71 \pm 0.11**
Ca (mg/dl)	7.59 \pm 0.38	7.34 \pm 0.46	6.12 \pm 0.42*	6.03 \pm 0.45**
Pi (mg/dl)	4.82 \pm 0.27	4.66 \pm 0.28	3.85 \pm 0.23**	3.22 \pm 0.22**
Na (mEq/L)	142.55 \pm 7.84	140.00 \pm 8.68	170.20 \pm 9.53**	188.03 \pm 12.16**
K (mEq/L)	4.96 \pm 0.27	4.68 \pm 0.24	3.89 \pm 0.23**	3.12 \pm 0.23**
T3 ($\mu\text{g/dl}$)	58.4 \pm 3.21	53.09 \pm 3.29	44.22 \pm 3.05**	42.88 \pm 3.13**
T4 ($\mu\text{g/dl}$)	4.88 \pm 0.27	4.25 \pm 0.26	3.62 \pm 0.25**	2.88 \pm 0.21**
TSH ($\mu\text{g/dl}$)	153.09 \pm 7.65	166.44 \pm 10.32	193.18 \pm 11.20**	213.55. 14.95**

* Significant at $P < 0.05$

** Highly significant at $P < 0.01$.

I-B- Pathological findings:**Thyroid glands:**

Grossly, the thyroid glands were markedly increased in size and it was pale, tan to grey in colour, soft and fleshy along the period of the experiment

Microscopically, the thyroid follicles showed vacuolar and hydropic degeneration, follicular cell hypertrophy and hyperplasia were evident with increased vascularity. Necrosis of some acini with leukocytic infiltration had been noticed among the acini at 7 days PA of ETU (Fig.1). While at 30 days, cystadenoma with a prominent fibrous capsule separating it from adjacent compressed thyroid parenchyma with leukocytic infiltration were noticed. The most characteristic features of these tumors were characterized by the extreme variability of size and shape of their follicles with diversity in cellularity and morphological characteristics of the follicular lining. The number of epithelial cells lining the follicles was significantly proliferate with hyperchromatic nucleus (papillary adenoma) (Fig.2). All of the follicles were devoid of colloid after 30 days. The thyroid of experimental rats which received the ETU at 90 days revealed cystadenoma and round cell infiltration with circulatory disturbances including congestion, thrombosis and hemorrhages, focal necrosis, mononuclear cells infiltration and fibrosis were the most prominent lesions (Fig.3). Other cases developed adenocarcinoma where the thyroid follicles showed hypercellularity and over lapped each other with deep

basophilic and hyperchromatic nuclei with mitotic activity, other revealed hypercellularity, pleomorphism and absence of basement membrane (Fig.4). Fine connective septa with capillaries subdivided the carcinoma into small lobules.

Liver:

Grossly, the liver was congested and enlarged with rounded borders and stretched capsule. Cut surface showed oozed blood at 7 days. While at 30 and 90 days, the liver was pale in colour, slightly firm in consistency with multiple small yellowish white foci.

Microscopically, at 7 days PA of the ETU, the liver revealed disarrangement of hepatic cord with hemorrhage. The bile duct showed cholangitis with congested blood vessels and pleomorphic nuclear leukocytes (Fig.5). At 30 days, the liver showed fatty change in hepatocytes and increase number of bile ductules with round cell infiltration. Some cases showed cholangioma in which, numerous irregular bile ductules of hyperplastic lining epithelium and scanty connected tissue together with congestion and round cell infiltration as well as coagulative necrosis of some hepatocytes (Fig.6). At 90 days, the hepatocytes showed several prominent nucleoli. The distribution of chromatin was very irregular, often being marginally displaced towards the nuclear membrane. Mitotic figures are numerous, and very often abnormal. The cytoplasm is usually acidophilic to a degree varying from weak to strong. Overall, the most prominent lesions in most cases were prephero-lobular fatty change centro-lobular coagulative necrosis (Fig.7).

Kidneys:

Grossly, the kidneys were dark red in colour and enlarged with stretched capsule at 7 days PA of ETU to the experimental rats. At 30 and 90 days, the kidneys were slightly pale in colour and moderately firm in consistency.

Microscopically, at 7 days PA of the ETU, the kidney showed acute focal interstitial nephritis in which, congested blood vessels, inflammatory cells in the interstitial tissue, nephrosis including fatty change, vacuolar degeneration and cloudy swelling in renal tubules were seen (Fig.8). At 30 days, coagulative necrosis in some renal tubules and edema under the Bowman's capsule with proliferation of mesangial cells of glomerular tuft were noticed. Other cases showed hemorrhage and edema in Bowman's capsule, necrosis in some renal tubules, hyaline casts, focal area of fibroblastic cell proliferation with round cells infiltration (Fig.9). Similar lesions were noticed but widely distributed after 90 days PA of the ETU to the rats, where acute focal interstitial nephritis in which degeneration in renal tubules with round cells infiltration, eosinophils with few fibroblastic cells proliferation had been detected (Fig.10). In some cases, the renal tubular epithelium exhibited excessive proliferation with glandular like masses. The neoplastic cells were pleomorphic with hyperchromatic nuclei and mitotic figures where other tubules were necrotic (Fig.11).

Stomach:

Macroscopically, the stomach in all sacrificed rats showed nodular thickening in non glandular part of stomach with focal cauliflower masses or thick diffuse masses.

Microscopically, at 7 days the non-glandular part of the stomach showed severe edema in lamina propria and among the muscle fibers with round cells infiltration. Focal destruction of the surface epithelium of keratinized part was seen. The glandular stomach showed congested blood vessels, edema in submucosa and aggregations of round cells among the gastric glands (Fig.12). After 30 days PA of the ETU to the experimented rats, the non glandular part showed squamous cell papilloma (acanthosis and hyperkeratosis) with focal aggregation of round cells under muscularis mucosa (Fig.13). While after 90 days PA of the ETU to the rats, the glandular portion showed round cells infiltration. The glandular epithelium had distinct glandular arrangement forming acini. The cells showed pleomorphism, hyperchromatic nuclei and numerous mitotic figures together with areas of hemorrhage and necrosis (Fig.14).

Intestine:

Macroscopically, the intestine in all sacrificed rats had thickened wall and slightly congested mucosa.

Microscopically, at 7 days PA of the ETU to the experimental rats, the lining epithelial cells of the intestinal villi showed epithelial metaplasia to goblet cells with partial desquamation of epithelial cells of intestinal villi beside numerous leukocytic infiltration in lamina propria and submucosa mainly round cells resulting in thickening of the intestinal villi (Fig.15). At 30 days, epithelial desquamation of intestinal villi with vacuolation in intestinal mucosa, and necrosis of intestinal glands with few round cells infiltration among the glands were also noticed (Fig.16). While after 90 days PA of the ETU to the experimented rats, in addition to the aforementioned lesions, the intestine showed infiltration of submucosa with pleomorphic cells and catarrhal enteritis. The glandular epithelium showed glandiform structures often irregular shape, lined with high cylindrical or cuboidal epithelium arranged in one or several rows. The basement membrane, in some glands, were broken

and cell nuclei were polymorphic, and differ in chromatin content, size, shape and position in respect of the longitudinal axis of the cell (Fig.17).

DISCUSSION

Ethylenethiourea (ETU) is a metabolite, environmental degradation product and minor technical impurity of the ethylenebisdithiocarbamate (EBDC) class of fungicides. Occupational exposure by dermal and inhalation routes may occur in the rubber and plastics industry and where ethylenebisdithiocarbamate fungicides are used. Individuals may be exposed to ethylene thiourea (ETU) through consumption of food contaminated with fungicides (released during cooking) [19, 20]

Our investigation revealed that, the clinical signs in rats exposed to ETU were nausea, ruffled or easily removed hair, Alopecia, unbalanced movement and significant decrease in food consumptions and body weight with marked swelling on both side of the neck were seen. Similar findings were reported [14, 15, 21, 22, 23].

Hematologically, clinical symptoms of anemia (decrease in RBCs, Hb and HCT) were detected in our study. Anemia has been associated with hypothyroidism [23] and was observed at termination in the 13-week inhalation study [24]. Our results in accordance with [22,23]. A significant decrease in WBCs in our study was seen. In the 13-week inhalation study [25], a similar significant decreased WBCs count due to lower numbers of circulating lymphocytes in male rats were also observed.

Regarding the clinical chemistry in our studies, the serum T₃ and T₄ were significantly decrease while the TSH were significantly increase and these in accordance with NTP,1992 it mentioned that the Serum levels of thyroxine (T₄) and/or triiodothyronine (T₃) were significantly decreased in rats receiving adult concentrations of 83 or 250 ppm, and thyrotropin (thyroid-stimulating hormone, TSH) was significantly increased at these concentrations. The recorded hypothyroidism, in the present experiment, could be attributed to inhibition of iodide uptake by the thyroid, thyroid peroxidase inhibition and damage to thyroid follicular cells [26].

The primary toxicological finding with ETU in laboratory animals is inhibition of the synthesis of thyroid hormones T₄ and T₃, leading to elevated serum levels of TSH in rodent [23, 26, 27, 28, 29, 30], via feedback stimulation of the hypothalamus and pituitary [31]. Prolonged and continuous elevation of serum TSH levels results in hypertrophy and hyperplasia of the thyroid follicular cells in rats, mice, monkeys, and dogs, and ultimately, in the development of nodular hyperplasia, adenoma, and/or carcinoma in rats and mice [6,32, 33, 34], but not hamsters [35]. There is evidence for reversibility [36, 37]. Direct evidence for inhibition of thyroid hormone synthesis by ETU has been obtained in rats *in vivo* [36,37,38]. ETU also reversibly inhibited thyroid peroxidase-catalyzed iodination reactions *in vitro* [39].

The significant increase in T-CHO, F-CHO and PL in our studies were in accordance with [22,40] whose reported that, the serum levels of phospholipid, total and free cholesterol were also increased significantly upon ETU and 2- Mercaptobenzimidazole administrations and these attributed to the thyroid hormones stimulate metabolism of cholesterol to bile acid and that hypercholesterolemia is characteristic of hypothyroid states [41].

Thyroid hormones are also known to participate in the regulation of the balance of intracellular and intercellular Na⁺ and K⁺ levels by active transport [42]. The serum Na⁺/K⁺ ratios in our study were markedly altered. This may be due to the severe hypothyroid state induced by ETU administration. [22] recorded that, a significant increase in Na⁺ and significant decrease in K⁺ in both males and females given 2-MBI.

Serum levels of Ca²⁺, Pi and ALP are altered by a parathyroid hormone imbalance which affects bone metabolism [43]. In our study, the serum levels of ALP, Pi and Ca²⁺ were significantly decreased. Similar finding reported by [23].

Increased blood clotting time (APTT), AST and ALT in our study attributed to the pathological alterations in liver. Slight but statistically significant increases of BUN and CRN were observed in our study, this in consideration of previous report demonstrating renal toxicity [25], and attributed to the present histopathological finding in kidneys.

The thyroid were severally enlarged, appear pale tan to gray in colour, soft and fleshy along the whole period of the experiment, similar findings were reported [22, 23].

Histopathologically, the rat's thyroid showed vacuolar and hydropic degeneration of its epithelial lining, necrosis, cystadenoma, papillary adenoma and adenocarcinomas with circulatory disturbances including thrombosis and hemorrhages together with fibrosis were the most prominent lesions. Such findings agreed with [44], who recorded that, one of zineb fungicide main metabolites, is ethylenethiourea (ETU), when orally administered to calves at 200 mg of zineb/kg of body weight for 80 days, had remarkable impairment of thyroid function, as reflected by reduction in serum concentrations of triiodothyronine and thyroxine and increase in weight of the thyroid gland associated with epithelial vacuolization and foci of hyperplasia. When rats fed 125 or 625 ppm ethylenethiourea for 90 days showed marked increases in serum thyroid stimulating hormone and high incidence of follicular carcinoma of the thyroid in rats after oral administration [21].

After 6 months administration of ethylenethiourea to dogs, the thyroid glands increased to approximately 30 times their normal weight and exhibited follicular-cell hypertrophy, hyperplasia and an increase of vascularity [45]. Most of the follicles had irregular shapes and varied greatly in size in addition to, the follicles often became lined by stratified epithelium.

Adenoma and adenocarcinoma in thyroid glands of rats were seen in our investigation, these findings were in agreement with finding of [32], they reported hypertrophy, hyperplasia, adenoma and adenocarcinoma of thyroid glands due to ETU in rats.

The toxic effects of ETU was studied in male and female rats receiving 83 or 250 ppm, the incidences of follicular cell hyperplasia or follicular cell adenoma of the thyroid gland were significantly increased relative to the controls [15]. The incidences of follicular cell carcinoma were significantly increased in the 250 ppm groups. Several studies have shown that, the prolonged administration of ETU to rats causes thyroid neoplasms [7, 27, 32, 46].

Ethylene thiourea induce thyroid tumors through the suppression of thyroxine syntheses, leading to hyperplasia of the thyroid gland. Such lesion may be attributed to the ability of ETU to cause hormonal disturbances. It interferes with the function of thyroid peroxidase resulting in a reduction in circulating thyroid hormone concentrations and increased secretion of thyroid stimulating hormone (TSH). TSH stimulates thyroid growth and thyroid hyperplasia which may be eventually develop into tumors [15]. ETU induced a statistically significant degree of DNA fragmentation in the thyroid. These findings suggest that, the tested compounds might be carcinogenic to thyroid in humans [47, 48].

Concerning liver lesions in our studies, similar findings were reported by [46, 49]. ETU produce an increase in liver weight and fatty degeneration in cells around central vein which were almost injured because they contain high concentration of cytochrom p 450 enzymes which is responsible for detoxification [50, 51].

Chronic ETU administration produces hepatocellular carcinoma in the mouse and rat [6, 32, 46]. The induction of liver neoplasms in mice exposed to dietary concentrations of 646 ppm ETU for 18 months was reported [6]. Two strains of mice fed ETU in the diet had an increased incidence of hepatomas [46]. Centrilobular hepatocellular cytomegaly occurred in male and female mice and rats receiving ETU at concentrations of 500 ppm or greater. Hepatocytes surrounding the central venules were enlarged, with homogeneously staining, finely granular eosinophilic cytoplasm [32].

The carcinogenic activity of ethylene thiourea in male and female B6C3F₁ mice as shown by increased incidences of thyroid follicular cell neoplasms, hepatocellular neoplasms, and adenomas of the pars distalis of the pituitary gland [15]. Non-neoplastic lesions associated with the administration of ethylene thiourea included follicular cell hyperplasia in rats and mice and follicular cell cytoplasmic vacuolation, centrilobular hepatocellular cytomegaly, and focal hyperplasia of the pars distalis of the pituitary gland in mice. Other effects associated with the administration of ethylene thiourea included decreased serum levels of T₄ and/or T₃ in rats and increased serum levels of TSH in rats.

Our pathological findings in thyroid and liver were in agreement with [14]. They reported that, thyroid carcinoma and hepatoma have been observed in rats and mice orally exposed to ETU.

The lesions in kidneys, intestine and stomach in our investigation were in agreement with the findings of [52], who reported tumors in different organs of rats (liver, kidney, thyroid, stomach and urinary bladder) due to ETU in drinking water. Similar findings were reported by [53]. Marked nephrosis and cancer detected in kidneys may be related to 90% of ETU and its metabolite is excreted primarily in the urine [51].

ETU is rapidly absorbed via the GI tract [54]. The elimination of ETU is largely renal [12], and its elimination half life is variable, ranging between 32-100 hr, according to the species involved [55]. EBDC residues accumulate preferentially in the thyroid, and secondarily in the liver [56].

ETU is one of carcinogenic compounds in animals especially rats. Although ETU has a weak genotoxic potentiality, it is able to induce chromosomal abrasion, cell transformation in mammalian cells and DNA fragmentation [57]. Also, it is a producers to a large amount of free radicals which responsible for formation of cancers and cells damages processes especially to lipids, protein and nucleic acids. This explains the high incidences of tumors as altered nodules, cholangioma in liver, adnecarcinoma in kidneys and squamous cells papiloma in stomach and adnecarcinoma in intestine.

The non-neoplastic lesions as degenerative changes and necrosis observed in different groups especially in liver, kidneys, stomach, intestine and thyroid gland were attributed to toxicity and free radical production by ETU. The exposure to free radicals induces changes in cells membrane which contribute to cell damage processes [58]. In addition to, the free radicals produced by ETU, tumor cells have the ability to produce substantial amount of hydrogen peroxide and reactive oxygen metabolites that are released into the circulation resulted in different degenerative changes which was obvious in different organs [59].

Although the present findings resulted from the exposure of rats to 500 ppm of ethylene thiourea (ETU) through a basal diet for 90 days, other experiments indicated that, ETU was used in different doses including 83 and 250 ppm [15] 60 mg/kg [35] 125 and 625 ppm [21] and proved that ETU were antithyroid substance even in low doses.

The EBDC metabolite ETU, have clear and important toxic effects in various animal species, and there is reason to at least suspect possible carcinogenicity of these agents in humans. Therefore, it is recommended that further human studies should be done to better define and assess this risk. Such research is feasible today, in light of available methods to quantities degrees of exposure to these chemicals. Furthermore, pending the results of such studies, it would clearly be desirable to undertake regular surveillance for humans exposed to EBDCs, with specific attention to markers of thyroid and hepatic pathology.

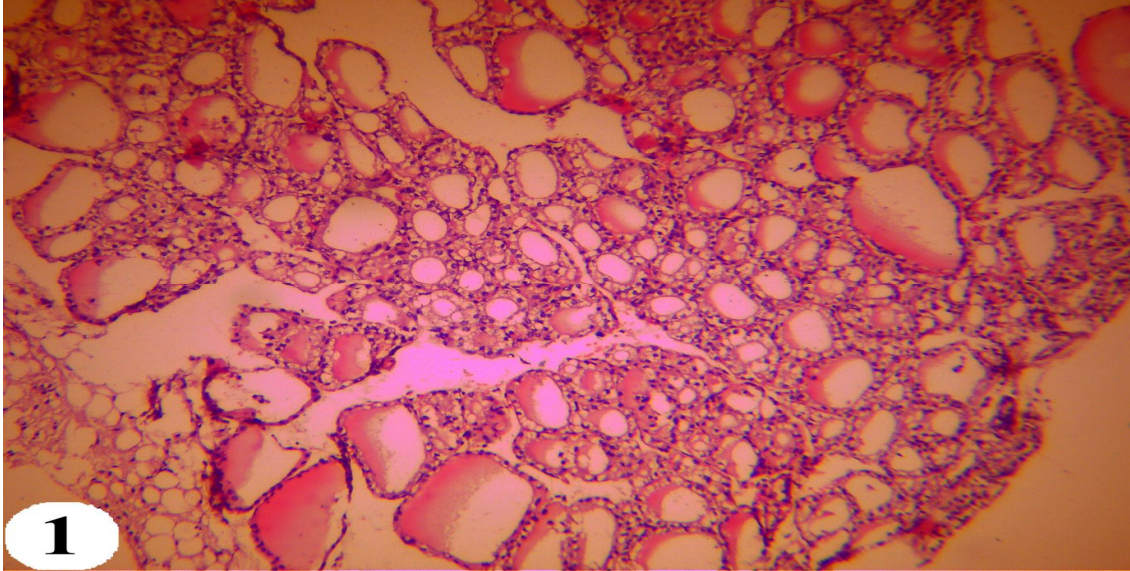


Fig. 1: The thyroid follicles, of rat at 7 days PA of ETU, showing vacuolar and hydropic degeneration with necrosis of some acini that infiltrated with leukocytic infiltration. H&E stain, X250.

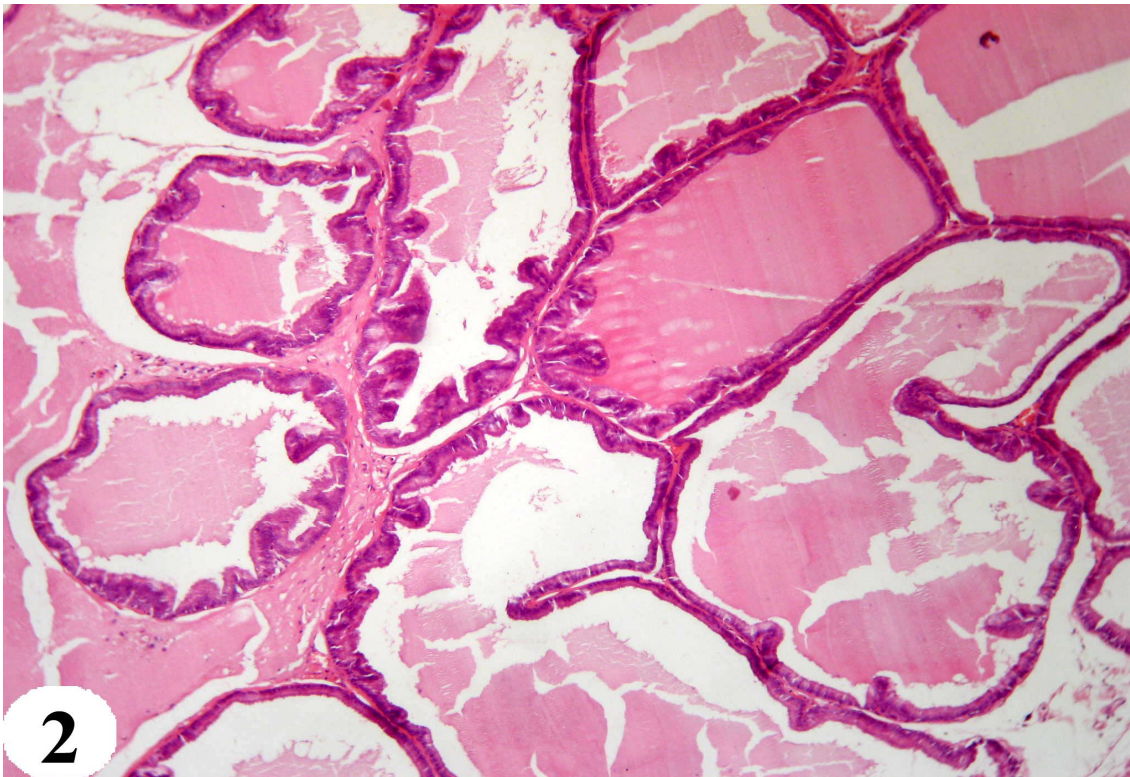
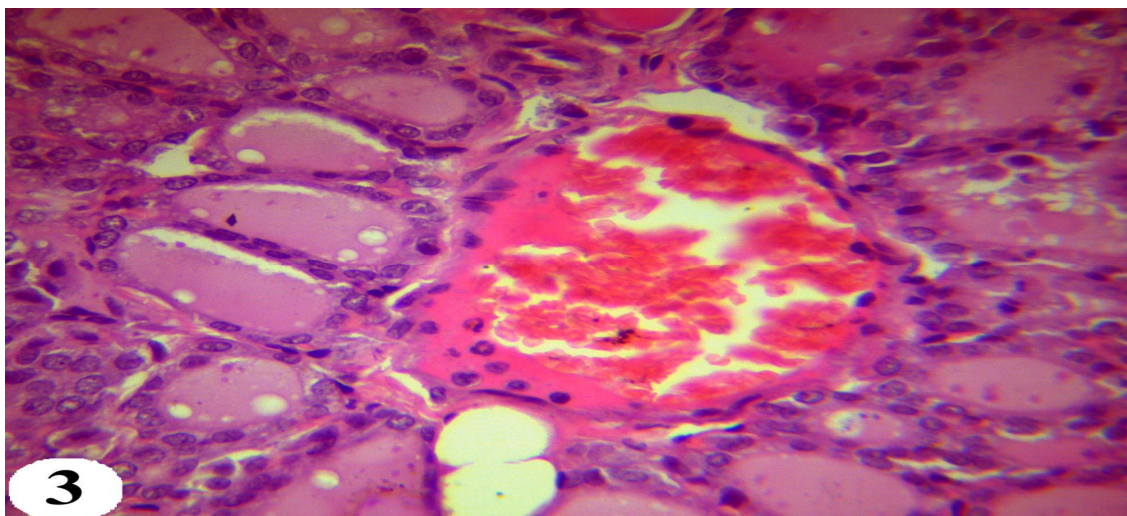
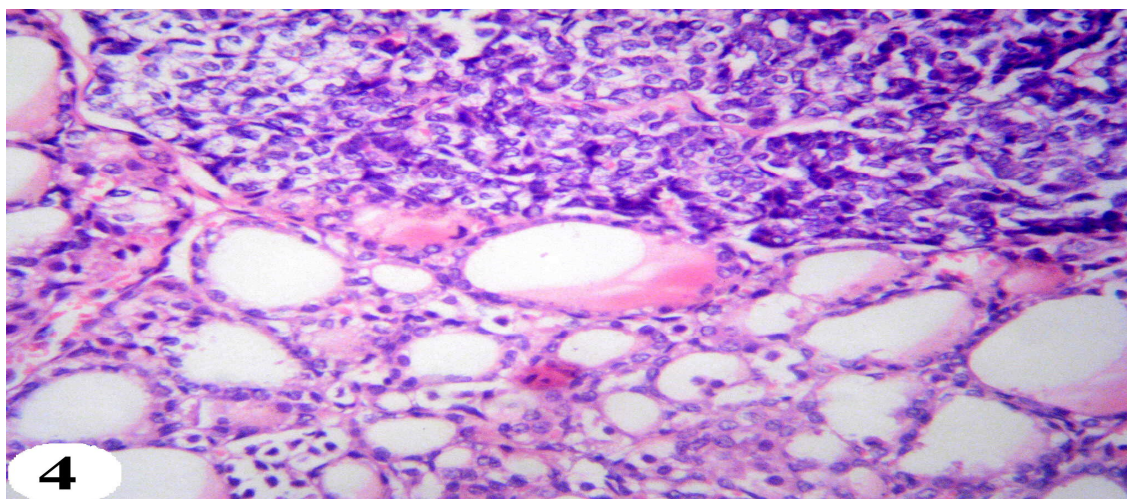


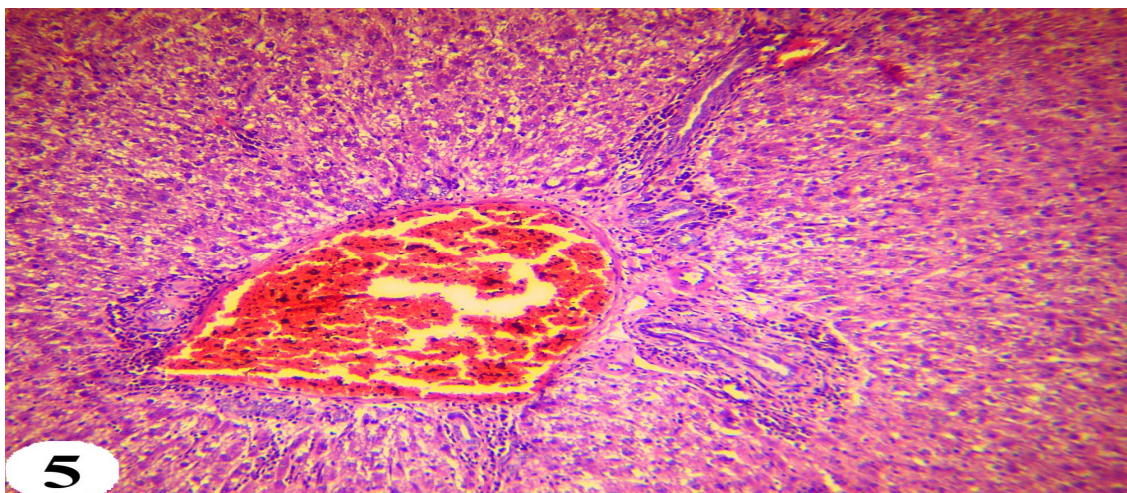
Fig. 2: The thyroid follicles, of rat at 30 days PA of ETU, showing papillary cystadenoma. H&E stain, X 100.



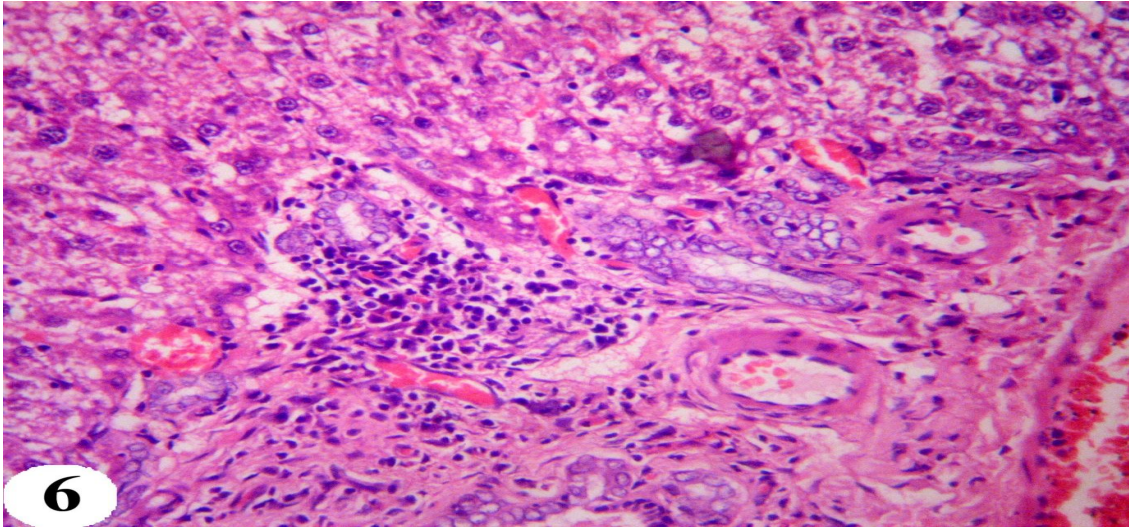
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Fig. 3: The thyroid follicles, of rat at 90 days PA of ETU, showing congestion, focal necrosis and mononuclear cells infiltration. H&E stain, X 250.



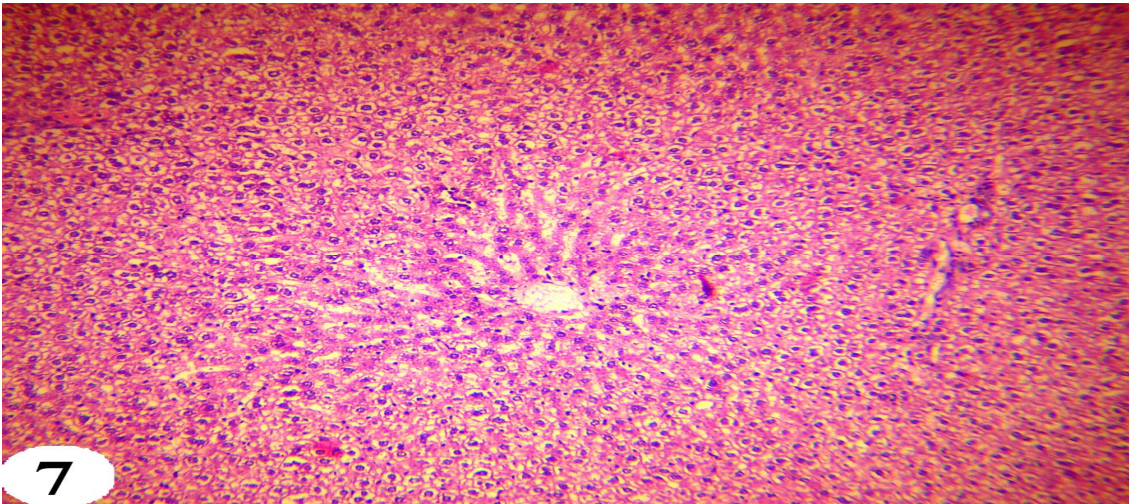
4
Fig.4: The thyroid follicles, of rat at 90 days PA of ETU, showing adenocarcinoma hypercellularity, deep basophilic cytoplasm and hyperchromatic nuclei with mitotic activity, along with absence of basement membrane. H&E stain, X 250.



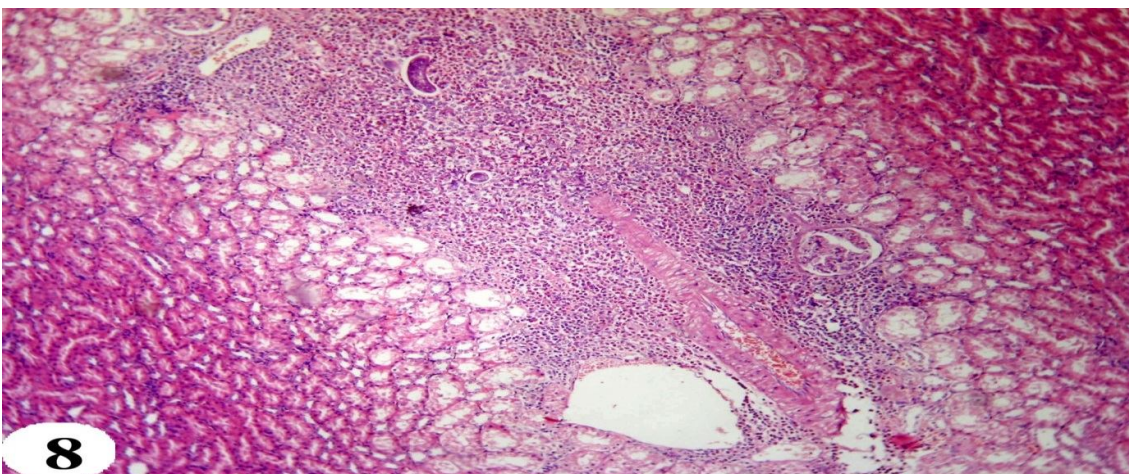
5
Fig. 5: Liver, of rat at 7 days PA of ETU, showing disarrangement of hepatic cord with haemorrhage and cholangitis represented by congestion and pleomorphnuclear leukocytes in the portal area. H&E stain, X 100.



6
Fig. 6: Liver, of rat at 30 days PA of ETU, showing fatty change in hepatocytes and increase number of bile ductules with congestion and round cell infiltration, cholangioma in which, numerous irregular bile ductules of hyperplastic lining epithelium and scanty connected tissue. H&E stain, X 250.



7
Fig. 7: Liver, of rat at 90 days PA of ETU, showing prehepatic fatty change and Centrolobular coagulative necrosis. H&E stain, X 100.



8
Fig.8 :Kidney, of rat at 7 days PA of the ETU, showing congestion and inflammatory cells in the interstitial tissue with tubular necrosis H&E stain, X 100.

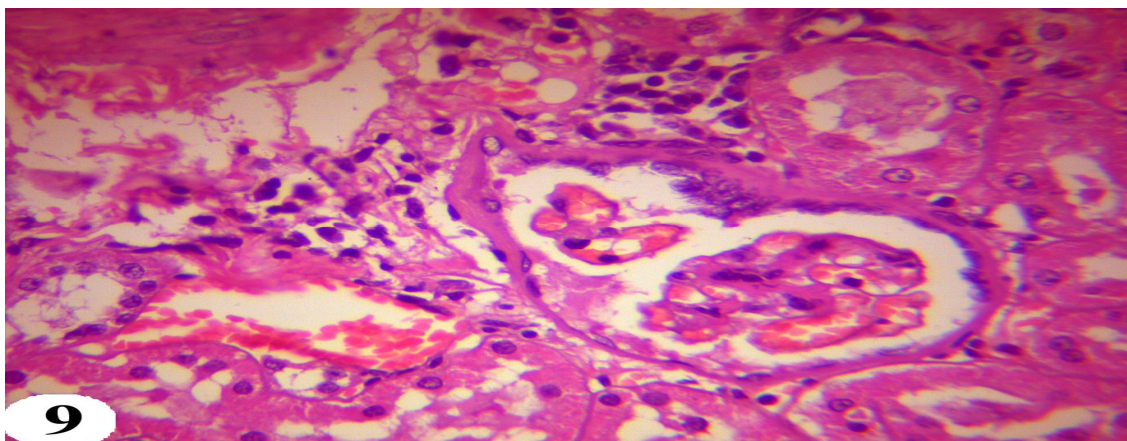


Fig. 9: Kidney, of rat at 30 days PA of the ETU, showing coagulative necrosis in some renal tubules with hyaline casts, congestion and round cells infiltration. Bowman's capsule was congested, edematous with proliferation of mesengial cells of glomerular tuft. H&E stain, X 250.

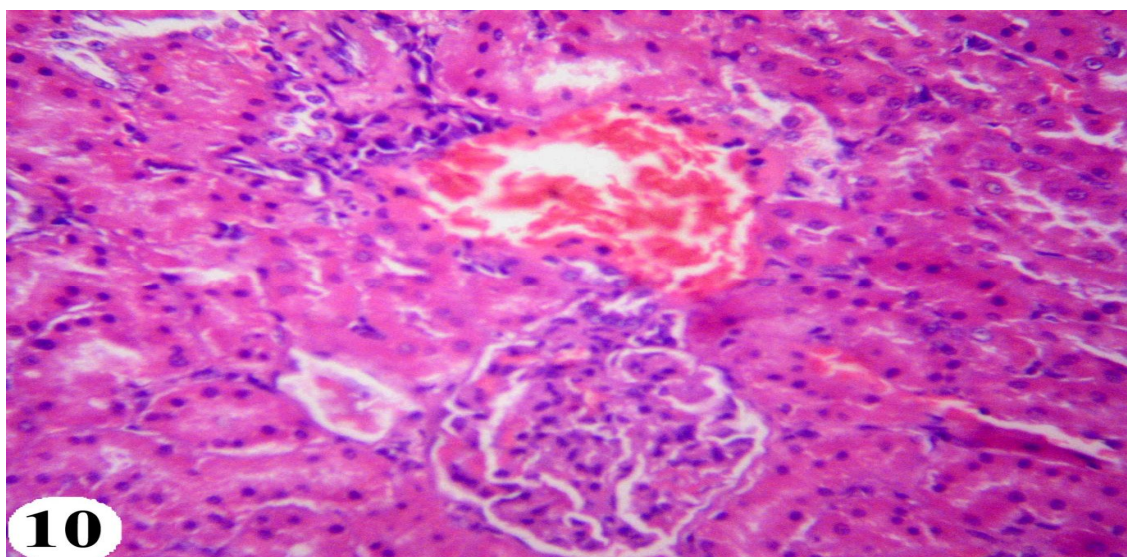


Fig. 10: Kidney, of rat at 90 days PA of the ETU, showing focal interstitial nephritis with degeneration in renal tubules and few fibroblastic cells proliferation. H&E stain, X 250.

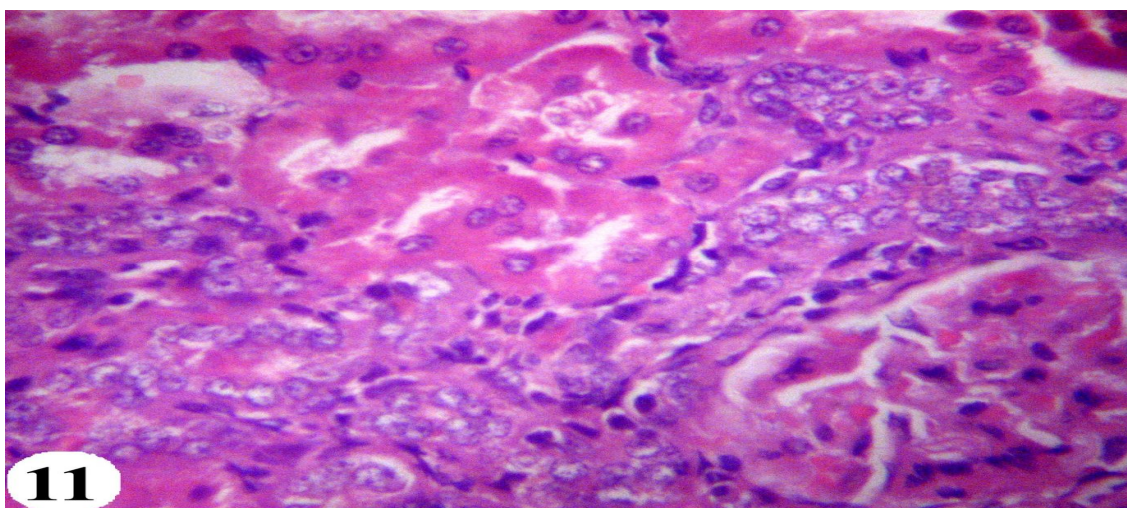
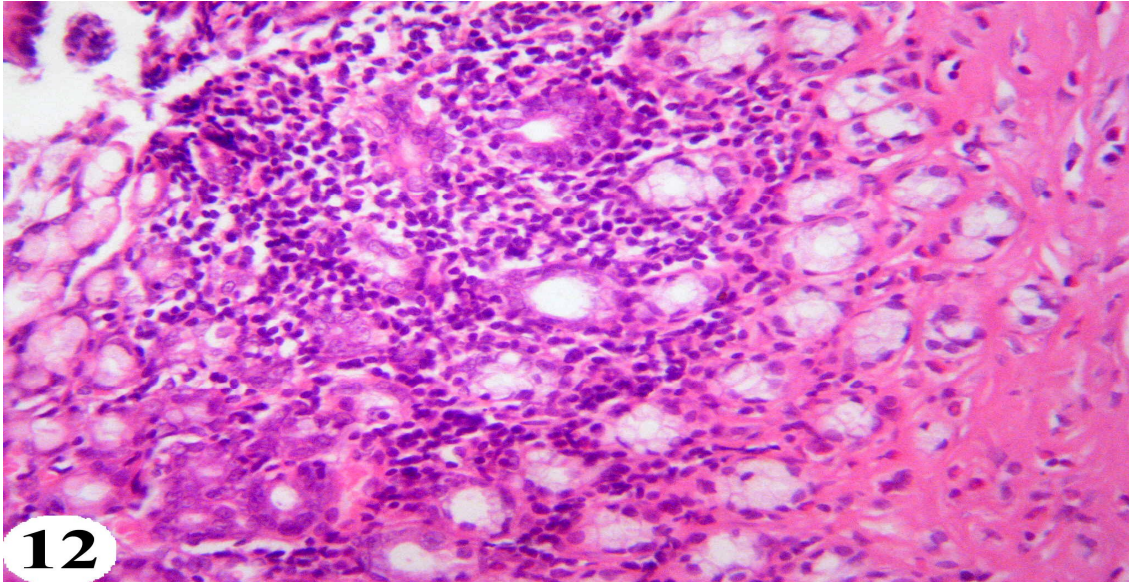
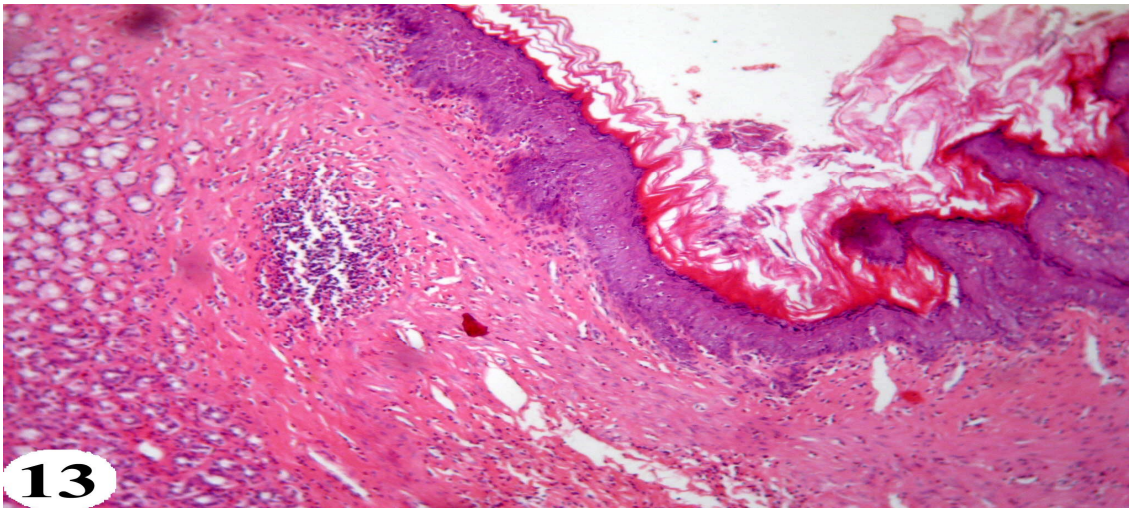


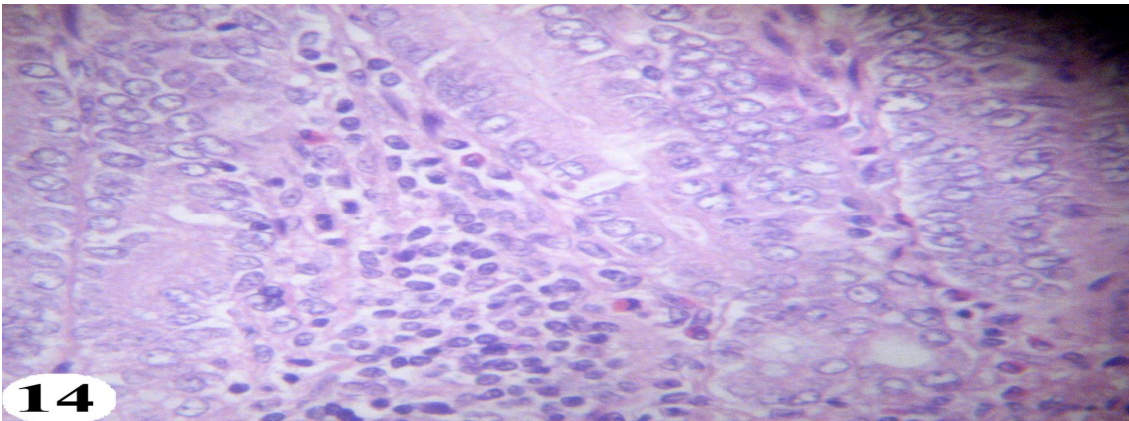
Fig. 11: Kidney, of rat at 90 days PA of the ETU, showing excessive proliferation of renal tubular epithelium with glandular like masses. Some cells were pleomorphic with hyperchromatic nuclei and mitotic figures while others were necrotic. H&E stain, X 250.



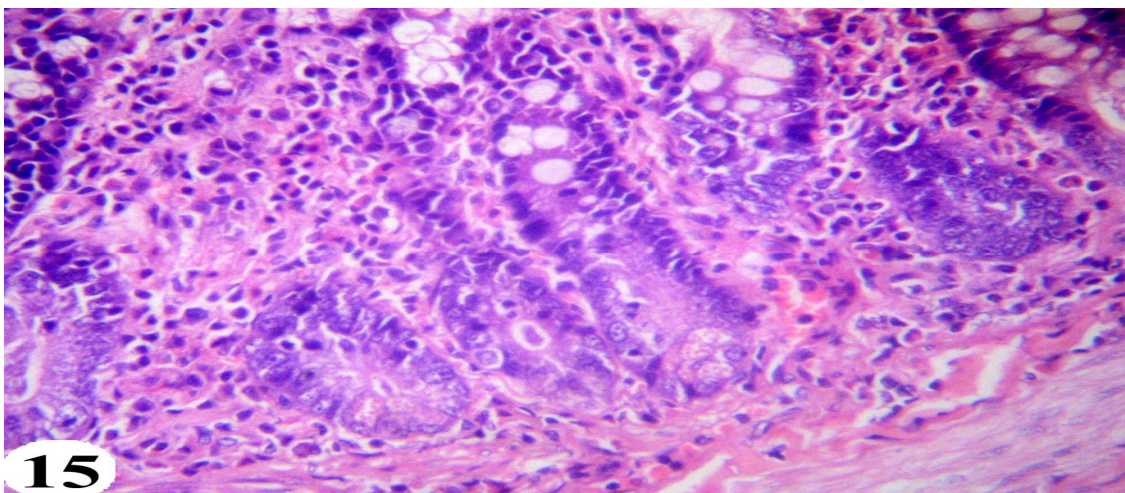
12
Fig. 12: Stomach, of rat at 7 days PA of the ETU, showing edema and aggregations of round cells among the gastric glands. H&E stain, X 250.



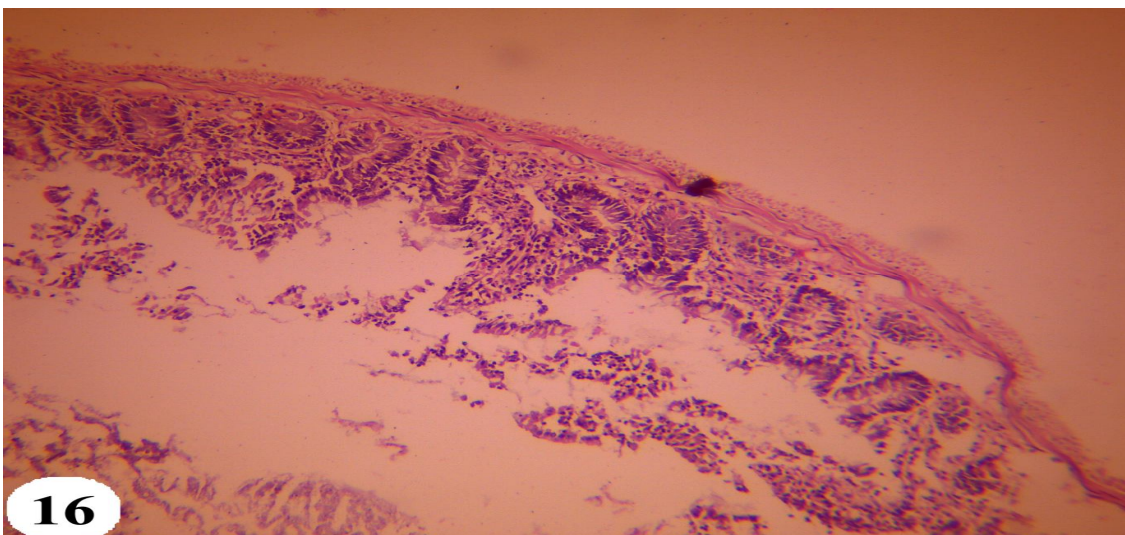
13
Fig. 13: Stomach, of rat at 7 days PA of the ETU, showing squamous cell papilloma (acanthosis and hyperkeratosis) with focal aggregation of round cells under muscularis mucosa. H&E stain, X 100.



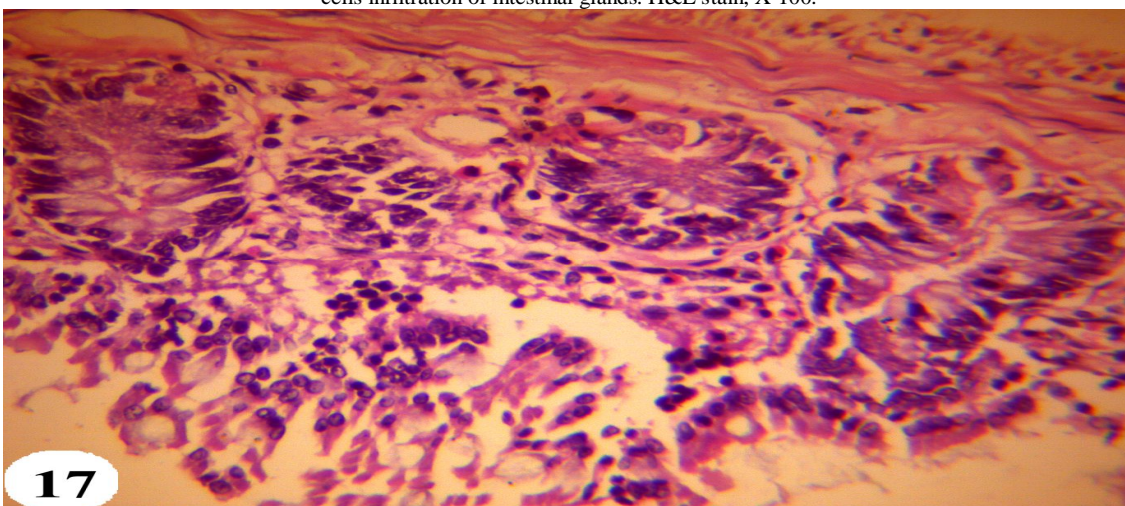
14
Fig. 14: Stomach, of rat at 7 days PA of the ETU, showing round cells infiltration between the glandular glands with distinct glandular arrangement forming acini. The cells showed pleomorphism, hyperchromatic nuclei and numerous mitotic figures. H&E stain, X 250.



15
Fig. 15: Intestine, of rat 7 days PA of the ETU, showing goblet cells with numerous leukocytic infiltration in lamina propria and submucosa. H&E stain, X 250.



16
Fig. 16: Intestine, of rat 7 days PA of the ETU, showing epithelial desquamation of intestinal villi with necrosis and round cells infiltration of intestinal glands. H&E stain, X 100.



17
Fig. 17: Intestine, of rat 7 days PA of the ETU, showing glandiform structures often irregular shape, lined with high cylindrical or cuboidal epithelium arranged in one or several rows. The basement membrane in some glands were broken and cell nuclei were polymorphic, and differ in chromatin content. H&E stain, X 250.

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